

Wiring Mechanisms for Olfaction and Vision—Not Completely Different after All

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Gradients of repulsive EphrinAs in the target were thought to repel temporal retinal ganglion cell axons expressing high levels of EphA receptors. Now, in this issue of *Neuron*, Suetterlin and Drescher (2014) show that EphrinA expressed on nasal axons contributes to the repulsion of temporal axons.

There is probably hardly any neuroscience graduate student who has not learned about the seminal work of Roger Sperry that led to the famous chemoaffinity hypothesis by which he proposed how the visual system gets wired (Sperry, 1963). According to his model, at least two perpendicular molecular gradients are necessary to identify each cell in a two-dimensional target. Thus, every target cell in the tectum carries a specific address label for incoming retinal ganglion cell (RGC) axons. By expressing the appropriate combination of receptors for these labels, RGC axons would be guided exactly to their target cells, resulting in the topographic map of the visual system that truthfully maintains the spatial information of the sensory input.

Specific targeting of projection neurons from the olfactory epithelium to the glomeruli in the olfactory bulb is of course also needed but in the olfactory system encoding of spatial information is not required. Rather, olfactory sensory neurons (OSNs) in the olfactory epithelium that respond to the same odorant converge in the same glomerulus of the olfactory bulb, forming a discrete rather than a topographic map (Cho et al., 2009). Thus, the olfactory and the visual systems are wired fundamentally differently (Figure 1A). Hence, it was not surprising that the molecular mechanisms underlying the wiring of these two systems were found to be different.

Classical in vitro experiments by Friedrich Bonhoeffer, the “Bonhoeffer stripe assay,” supported Sperry’s chemoaffinity hypothesis and demonstrated a gradient of repulsive molecules along the anterior-posterior axis in the chicken tectum as the driving force behind the

distinct topographic pattern of RGC axon targeting: nasal axons innervate the posterior tectum because they are less sensitive to the repellents, whereas temporal axons are restricted to the anterior tectum (summarized by Weth et al., 2014; Figure 1B). Finally, some 30 years after Sperry’s chemoaffinity hypothesis was published, these experiments led to the discovery of Ephrins (Drescher et al., 1995; Cheng et al., 1995) and Eph receptors (for a review, see Lisabeth et al., 2013). Eph receptors and Ephrins can be subdivided into two groups each (Lisabeth et al., 2013). EphA receptors bind to glycosyl-phosphatidyl-inositol-anchored EphrinAs, whereas EphB receptors bind transmembrane EphrinBs. The human genome encodes nine EphA and five EphB receptors, five EphrinAs, and three EphrinBs. EphrinAs and EphA receptors were found to be responsible for the rostrocaudal mapping, whereas EphrinBs and EphBs were shown to be required for lateral-medial mapping of RGC axons in the tectum (summarized in Weth et al., 2014).

EphrinAs are expressed in an anterior-low-posterior^{high} gradient in the tectum (Figure 1C). Their receptors, in particular EphA3, are expressed in a nasal^{low}-temporal^{high} gradient in the retina. Thus, RGC axon targeting in the tectum was explained by increasing repulsion of axons expressing higher receptor levels from more repulsive posterior areas in the tectum due to higher ligand levels: nasal axons can extend into the posterior tectum, as they express low receptor levels, whereas temporal axons are repelled more strongly from the same areas and therefore remain confined to the anterior part of the tectum. The

behavior of RGC axons in vivo mirrored the observed behavior of axons in the Bonhoeffer stripe assay, where nasal RGC axons were seen to grow on stripes containing membrane preparations taken from the anterior and the posterior tectum. In contrast, temporal RGC axons only extended on stripes containing membranes from the rostral tectum (Figure 1B).

However, why axons expressing EphA receptors would innervate the tectum at all remains an unresolved issue. Initially, the idea was that attractive cues expressed by the tectum could explain the ingrowth of axons into the tectum, while the repulsive system set up by Eph receptors and repulsive Ephrin ligands would then be sufficient to explain the distribution of nasal and temporal axons within the tectum. However, to date no such driving force or attractive cue has been identified. Instead, further studies identified countergradients of EphA receptors and EphrinA ligands also in the tectum and in the retina, respectively (summarized by Suetterlin et al., 2012). Based on these findings, *cis*-interactions between EphA receptors and EphrinA ligands on RGC axons were suggested to fine tune their sensitivity to the repulsive environment in the tectum.

This model was also compatible with studies demonstrating that absolute levels of EphA receptors and EphrinA ligands were not important, as axons were found to be distributed in the tectum based on relative levels of Ephrins (Brown et al., 2000). These studies were in agreement with Sperry’s observations that removal of one half of the retina would not result in a partially innervated tectum and conversely that removal of half of

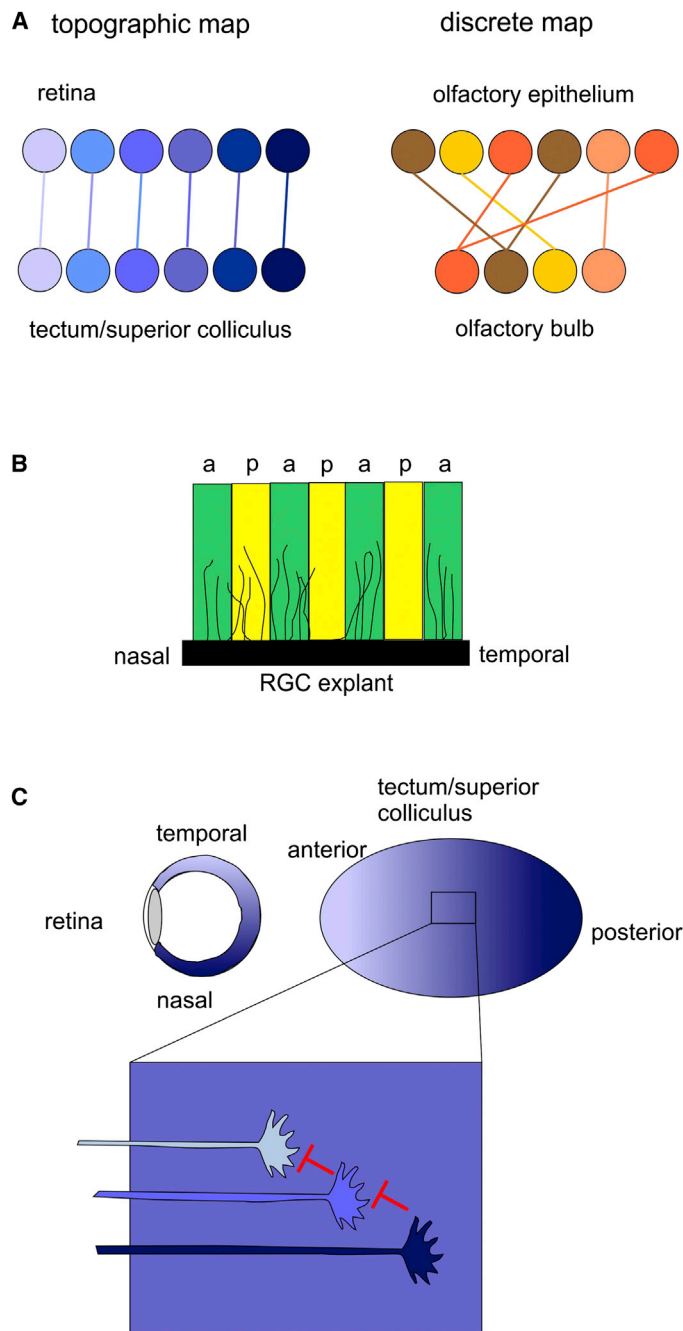


Figure 1. Map Formation Requires Axon-Target and Axon-Axon Interactions

(A) The wiring pattern of the visual and the olfactory system differ fundamentally. In the visual system, projection neurons from the retina, the retinal ganglion cells, maintain their spatial order in the target area, the tectum in nonmammalian vertebrates, and the superior colliculus in mammals. In the olfactory system, olfactory sensory neurons that respond to the same odorant converge onto the same glomerulus in the olfactory bulb. In the more recent literature, the term topographic map has unfortunately also been used for the olfactory system to indicate that the location of glomeruli in the olfactory bulb has some spatial organization. However, this should not be confused with the topographic map in the visual system, where neurons in the retina maintain their spatial organization in the target area, such that axons from neighboring RGCs target neighboring tectal cells to maintain the visual input.

(B) For the Bonhoeffer stripe assay membranes of anterior (a) and posterior (p) tectum are prepared separately and offered as substratum for retinal explants (RGC explant) in a striped pattern. Nasal axons are insensitive to the repulsive activity expressed in the posterior tectum and grow on either type of stripes, whereas temporal axons fail to grow on posterior tectal membranes.

the tectum would still preserve the topographic pattern of innervation in the remaining tectum. Thus, the prevailing idea is that RGC axons read the relative repulsive strength of the tectum and home in on an anterior-posterior position that is compatible with their active EphA expression level (Suetterlin et al., 2012).

Based on all these studies, topographic map formation was considered to be the result of RGC axon-target interaction with a competition between RGC axons for less repulsive anterior positions in the tectum. However, based on a detailed comparison of retinotectal mapping in different species during development and regeneration of the visual system and by considering observations made in vitro by the Bonhoeffer lab, Weth and colleagues recently suggested a new model for visual system wiring. Based on their observations and theoretical considerations, they postulated that axon-axon interactions would contribute to topographic mapping (Weth et al., 2014).

The paper by Suetterlin and Drescher (2014) now provides experimental support for the hypothesis that axon-axon interactions contribute to topographic map formation. The authors used conditional knockout mice lacking EphrinA5 either in the retina or in the superior colliculus, the target for RGC axons in mammals, to revisit observations made by Friedrich Bonhoeffer and colleagues a long time ago (summarized by Weth et al., 2014; Suetterlin and Drescher, 2014). They concentrated on EphrinA5 because it is expressed in a strong gradient in the retina, in contrast to EphrinA2 and EphrinA3, which are found to be expressed in a shallow gradient or uniformly. The comparative analyses of two populations of RGC axons targeting in the central area of the superior colliculus demonstrated that axon-axon interactions are crucial for the proper targeting of axons along the anterior-posterior axis.

They found that temporal axons innervating a central area of the superior colliculus (SC) were only slightly affected in

(C) A novel study (Suetterlin and Drescher, 2014) demonstrates axon-axon interactions as a mechanism contributing to topographic mapping in the superior colliculus at a local scale. Nasal axons expressing high levels of EphrinA5 repel temporal axons and thus support the global response derived from axon-target interaction.

their targeting in mice lacking EphrinA5 either in the colliculus or in the retina. However, a strong defect in temporal RGC axon targeting was found in mice lacking EphrinA5 in both retina and colliculus. In this case, temporal RGC axons formed ectopic termination zones at more caudal positions, as they were no longer repelled by the target and by nasal axons.

In contrast, nasal RGC axons were not affected more strongly in mice deficient in EphrinA5 in both retina and SC compared to mice lacking EphrinA5 only in the SC. Thus, EphrinA5 in the retina appeared not to contribute to the targeting of nasal RGC axons. Together with observations made for nasal and temporal RGC axons that were innervating more peripheral areas of the SC, [Suetterlin and Drescher \(2014\)](#) concluded that the caudal overshooting of temporal RGC axons in mice lacking EphrinA5 in both retina and SC was due to the absence of repellent axon-axon interactions.

These findings not only provide experimental evidence for the proposed model by [Weth et al. \(2014\)](#) but also shed some new light on the theory that *cis*-interactions between EphA and EphrinAs would fine tune the sensitivity of RGC axons ([Hornberger et al., 1999](#)). The observed axon-axon interactions would require EphrinA expression on RGC axons to repel other RGC axons expressing EphA receptors by *trans*-interactions and, thus, compete locally for target cells in the tectum or the superior colliculus, respectively.

Finally, the detection of axon-axon interactions as a mechanism contributing to topographic mapping brings the visual

system closer to the olfactory system. In the olfactory system, axons do not need to maintain any spatial information when innervating their target. Rather cells responding to the same sensory stimulus or odorant innervate the same glomerulus in the olfactory bulb. The molecular mechanism underlying the convergence of axons is largely unknown. Instead of the classical axon guidance cues identified in other systems, the olfactory receptors themselves were suggested to be responsible for olfactory sensory neuron axon guidance to the olfactory bulb. A few years ago, axon-axon interactions before innervation of the olfactory bulb were shown to result in a global distribution of OSN axons in the olfactory bulb ([Imai et al., 2009](#)). Axons innervating different areas of the olfactory bulb did not intermingle due to the expression of a secreted repulsive signal or its receptor, respectively. A contribution of axon-axon interactions to the innervation of the olfactory bulb is not a special feature of the olfactory system, as axon-axon interactions prior to target innervation were found previously also for muscle innervation by sensory and motor axons, for instance ([Milner et al., 1998](#)).

With their findings of axon-axon interaction as an important mechanism contributing to topographic map formation, [Suetterlin and Drescher \(2014\)](#) have shown that the visual system is not so different from other systems after all. Based on these new results, the mechanisms underlying the formation of a discrete and a topographic map are no longer completely different.

Some differences remain, however. In the olfactory system, axon-axon inter-

actions were found to be important before contact with the target. Furthermore, axon-axon interactions in the olfactory system are required for global patterning, whereas interactions between RGC axons in the visual system are important locally to sort out axonal topography.

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